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(57) Abstract

The invention relates to lymphokine activated cells, therapeutic compositions including such cells, methods of producing lymphokine activated cells and to methods of treatment involving such cells. The activated cells are useful in methods of treating infectious diseases, such as AIDS and ARC.

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Lymphokine activation of cells for adoptive immunotherapy, eg. of HIV infection.

DESCRIPTION

The present invention relates to lymphokine activated cells, therapeutic compositions including such cells, methods of producing lymphokine activated cells and to methods of treatment involving such cells.

Adoptive immunotherapy is a process whereby the body's immune system is reinforced by therapeutic means. In one known such method lymphocytes are withdrawn from a patient, fractionated, purified and then treated with a lymphokine.

Lymphocytes activated by treatment with a lymphokine are known as lymphokine activated killer (LAK) cells and are known to have greatly enhanced activity. Suitable lymphokines for use in any aspect of the present invention, include alpha interferon, gamma interferon, interleukin 1, interleukin 2 and interleukin 2 produced by phytohaemagglutinin (PHA).

After activation the LAK cells are used into the patient.

Such a process has been shown to provide clinical improvements in some patients but suffers from a number of disadvantages. In the first place it is of limited value where the patient's lymphocytes are severely damaged, such as may occur in HIV positive patients, or in severe oncologic disease.

Further, the process is time consuming and requires skilled laboratory work if it is to be effective. We have found that in some circumstances the methodology can be substantially abbreviated, thereby making its benefits more generally available.

According, therefore, to a first aspect of this invention there is provided a method for the activation of lymphocytes from a patient or donor for use in adoptive immunotherapy characterised in that autologous or donor lymphocyte containing body liquid is incubated with a lymphokine; whereby the resultant culture containing LAK cells is returnable directly to the patient.

A second aspect of the invention provides a reinfusable therapeutic composition for use in adoptive immunotherapy which comprises an autologous or donor (allogenic) lymphocyte containing body liquid activated by a lymphokine to provide patient-specific LAK cells.

In a third aspect of the invention there is provided a therapeutic composition for use in adoptive immunotherapy, which composition comprises autologous lymphocytes activated with a lymphokine to form lymphokine activated killer cells (LAK cells) for reinfusion into a patient;
characterised in that said composition additionally comprises lymphokine activated donor lymphocytes.

A fourth aspect of the invention provides a method of activating donor lymphocytes for use in adoptive immunotherapy, which method comprises, incubating said donor lymphocytes with a lymphokine to give LAK cells, characterised by preparing said LAK cells for direct infusion into a patient.

A fifth aspect of the present invention provides a method for activating autologous lymphocytes for use in adoptive immunotherapy, which method comprises incubating said autologous lymphocytes with a lymphokine to give LAK cells;
characterised in that donor lymphocytes are incubated with a lymphokine and the admixture of autologous and donor LAK cells is prepared for reinfusion into a patient.

Preferably the lymphocytes are fractionated after incubation with the lymphokine.

The lymphocytes are collected from a patient to be treated. Such a collection may be by lymphopheresis or, where lymphopheresis is impractical, by straight venesection.

The activation of the lymphocytes is initiated before any fractionation thereof. A lymphokine, for example interferon alpha, or gamma, or interleukin 1, or 2 is added to the total collected material, whether this is pheresed cells or whole blood. Where the patient to be treated is HIV positive, azidotymidine

(AZT) is added to the lymphocytes to prevent viral replication. The suspension so formed is incubated with the selected lymphokine.

In the first aspect of the invention the LAK cell containing culture is then immediately reinfused into the patient. Where, however, the patient's lymphocytes are damaged beyond a certain degree, further steps are necessary.

Accordingly, the lymphocytes are separated from the mother liquor to give a percentage of lymphocytes of greater than 90% and preferably greater than 95%, having a similar percentage viability.

The lymphocytes are then washed with a buffer solution, preferably on a serial basis and are then suspended in a suitable culture medium, for example, RPMI. The so-formed culture is then incubated for a suitable period, for example, 48 to 72 hours. After incubation, the culture is subjected to centrifugation to separate off the supernatant portion containing the LAK cells for reinfusion. These are purified prior to reinfusion into the patient.

In this latter process donor's cells, from a party such as a patient's relative, are formed in precisely analagous way with the exception, of course, that the addition of AZT will not be required. This provides a reservoir of healthy LAK cells which may be utilised to potentiate the activity of autologous LAK cells. The donor LAK cells are added, for example, in an amount of 1:2 to 1:4 to the patient's LAK cells prior to a final purification and preparation step.

In a sixth aspect, the present invention relates to a method of treating a patient suffering from an infectious, or oncological disease, comprising infusing LAK cells activated by a method in accordance with any of the first, fourth or fifth aspects of the invention; or with a therapeuctic composition in accordance with the second or third aspect of the invention.

Advantageously, the method comprises the step of firstly isolating the cells for activation from the patient to be treated, or another individual. The cells for activation may be a mixture of cells from both said sources.

Human immune deficiency virus (HIV) is the etiological pathogen in the development of the acquired immune deficiency syndrom (AIDS). The virus infects and destroys activated cells of the immune system leading to a profound defect in the host's natural immunity.

In recent years several anti-viral agents have been investigated for experimental treatment of AIDS, but no treatment has been found to cure the disease.

A vaccine to prevent worldwide spread of the disease is not at present obtainable. Within 6 years since the virus was identified, more than 50,000 cases have been reported to the World Health Organisation. The spread of the disease among heterosexual populations is still increasing. Thus, treatments that halt the disease, if not to cure it, are urgently required.

It is believed that for a retro-virus such as HIV to replicate, the virus must bind to a receptor site on the surface of a "host" cell and thereafter enter the cytoplasm of the host cell. The virus then causes the insertion of its "foreign" genetic material into the host cell's genetic material; with the result that, the

host cell is caused to produce fresh virus and eventually to perish. Additionally, the normal function of the host cell is often impaired and the host cell may age prematurely.

In an individual infected with HIV, the virus replicates by binding to a CD4 receptor site on the surface of a cell having such a site, for example a T-helper cell (a species of lymphocyte), and, thereafter entering the cell and inserting foreign genetic material into that of the T-helper cell. On entering a cell's cytoplasm HIV sheds its envelope and exposes its core RNA. A portion of DNA, corresponding to the viral RNA, is then inserted into the host cell's DNA by enzymatic action. The host cell's mechanism then begins to produce fresh HIV on the 'instructions' of the DNA introduced by the infecting virus. The fresh HIV is then expressed out through the infected cell's wall and into the circulation, where the fresh virus may infect further cells. The virus is able to remain in the host cell's cytoplasm, prior to shedding its envelope, for a considerable period of time and, hence, effectively lie dormant, safe from antibodies thereto

in the circulation. T-helper cells infected in this manner have impaired performance and age prematurely.

HIV is able to enter a cell having a CD4 receptor within less than 1/2 hour, which is not sufficient time for the immune system to respond to the presence of the virus and produce antibodies thereto.

The infected cells act as reservoirs of virus, although they may be destroyed by the action of the immune system once it has learnt to respond to the virus, when they are actually expressing HIV. The response of the immune system to HIV and other opportunistic infections, which give the characteristic symptoms of AIDS and AIDS related complex (ARC), is impaired because the Helper cell population is reduced, both by the cytopathic effect of HIV and the action of the immune system's killer cells destroying infected helper cells.

According to a seventh aspect of the present invention, there is provided a composition, comprising lymphokine activated cells having binding sites for an infectious agent characterised in that said lymphokine activated

cells have been modified after activation so as to be unable to facilitate virus reproduction. Preferably the infectious agent is a virus, more preferably it is a retro-virus and most preferably HIV. The cells may be fractionated or purified before or after treatment with a lymphokine.

In an embodiment of the seventh aspect of the invention, the activated cells are lymphocytes. It is believed that when a lymphocyte is activated with a lymphokine, the number of suitable binding sites for HIV on the surface of the resulting lymphokine activated cell, is greatly increased over that on a normal resting lymphocyte. Thus, HIV is more likely to bind with an activated lymphocyte than a normal resting lymphocyte. Accordingly, when introduced into the circulation of an individual infected with HIV, activated lymphocytes bind to free virus in preference to normal non-activated lymphocytes. Since an activated cell in accordance with the present invention cannot reproduce virus, any viral genetic material introduced into such a cell by a virus which has entered that cell, is ineffective. Accordingly, once a virus has entered an activated cell in accordance with

the seventh aspect of the present invention, that virus is effectively eliminated from the circulation and prevented from replicating.

In a further embodiment of the seventh aspect of the invention, modification of the activated cells involves the disruption, or destruction of the cells' genetic material. Preferably, this modification is achieved through the use of a chemical agent such as a DNA inhibitor, which prevents reproduction, but does not interfere with normal cell function. A suitable DNA inhibitor is mitomycin-C. Alternatively modification may be achieved through the irradiation of the activated cells. Thus, when HIV or a like virus binds to and enters such an activated cell and introduces its genetic material into said cell, either the virus is unable to cause the insertion of foreign genetic material into the cell's material, or replication of virus by such material is prevented and, accordingly, fresh virus cannot be produced. Lymphocytes for producing activated cells in accordance with the seventh aspect of the present invention may be derived from a healthy donor, both directly or indirectly, or they may be produced by an established cell line, such

as CEM or H9 (these originate from T-Helper cells and are commercially available). Most preferably, the lymphocytes include T-helper cells. Alternatively, the lymphocytes may be derived from cell lines which have been 'improved' by recombinant techniques to have additional receptor sites.

In an eighth aspect, the present invention provides a process for activating and modifying cells having binding sites for an infectious agent comprising treating said cells with a lymphokine and, subsequently, modifying the resultant activated cells in order to render virus replication impossible in said cells, while maintaining the cells' ability to bind said infectious agent. Preferably the infectious agent is a virus, more preferably it is a retro-virus and most preferably it is HIV.

In an embodiment the cells for modification are lymphocytes.

In a further embodiment of the eighth aspect of the invention, modification of the activated cells involves the disruption or destruction of the cells genetic

material. Preferably, this modification is achieved through the use of a chemical agent, for example a DNA inhibitor such as mitomycin-C, or through the irradiation of the activated cells.

In a preferred embodiment of the eighth aspect of the invention, lymphocytes for producing the activated cells are derived from a healthy donor either directly or indirectly, or they may be derived from an established cell line, such CEM or H9. Preferably, the lymphocytes include T-helper cells. The lymphocytes may be derived from cell lines which have been 'improved' by recombinant techniques to have additional receptor sites.

In a ninth aspect of the present invention there is provided a therapeutic composition, for use in the treatment of infection with an infectious agent including HIV infection or AIDS, comprising activated cells in accordance with the seventh aspect of the invention, or cells processed in accordance with the eighth aspect of the present invention.

In a tenth aspect of the present invention there is provided a method of treating a human patient infected with an infectious agent, said method comprising infusing a composition in accordance with the ninth aspect of the present invention into said patient. The infectious agent may be a virus, is preferably a retro-virus and more preferably HIV.

It is believed that when the activated cells in accordance with the seventh aspect of the present invention enter the circulation, these cells bind to HIV present therein, to thereby reduce the level of free HIV in the patient's blood stream. Since any HIV which binds to an activated cell in accordance with the seventh aspect of the present invention is effectively destroyed, the ninth aspect of the present invention has the effect of filtering virus from a patient.

Activated cells in accordance with the seventh aspect of the present invention only survive for a period of 1/2 hour to about 5 days, because of their non-functioning genetic material. Therefore, these cells are quickly cleared from a patient's system after they have performed their task.

In accordance with an eleventh aspect of the present invention a process for reducing the concentration of an infectious agent such as HIV, or a like virus, in infected plasma comprises, incubating said plasma with a composition in accordance with the seventh aspect of the present invention. In an embodiment, activated cells in accordance with the seventh aspect of the present invention are removed from said plasma after binding with HIV, or a like virus. Preferably, this removal is effected by centrifuging the plasma and inventive composition mixture after an incubation period of 1-24 hours. Plasma so treated may be returned to a patient in a form having a much reduced level of HIV and substantially no activated cells, or remains thereof.

It is believed that healthy lymphocytes are stimulated to respond to the presence of an antigen, exemplified by an infectious agent such as virus, when exposed to said antigen. Thus, for example, when a person becomes infected with a strain of the common cold virus, his immune system is firstly stimulated by the presence of the virus and, once so stimulated, it responds to the presence of the virus and eliminates it. During the

process of stimulation prior to response, the virus population expands rapidly, so that the infected individual exhibits the symptoms of a cold; but when the immune system responds, these symptoms recede as the viral population is decimated by the responsive action of the immune system.

An individual's immune system may also be taught to respond to the presence of a harmful virus by prior stimulation with incomplete, or dead virus of the same type, or with a virus of a similar, but less harmful type such as a genetically engineered virus. This process is the well known vaccination process and its advantage is that an individual's immune system, after vaccination, will respond rapidly to an invasion by a harmful virus, to eliminate that virus before it has a chance of multiplying sufficiently to cause harm. Otherwise, without vaccination, a harmful virus can multiply sufficiently to harm, or even kill, before an infected individual's immune system has time to be stimulated and to respond. Vaccination also prevents secondary infection with the same virus and thus gives long lasting protection.

It is believed that, for an individual infected with an infectious agent, for example with a retro-virus such as HIV, to remain outwardly substantially healthy and exhibit little or none of the symptoms of infection, his lymphocytes must be capable of producing sufficient and effective antibodies to control the replication of the infectious agent. That is, the amount of said infectious agent within such a patient's body is kept at a sufficiently low level, by the action of this immune system, for the infectious agent to have little or no deleterious effect.

In accordance with the present invention, in a twelfth aspect, there is provided a process for activating and stimulating lymphocytes to respond to an infectious agent, comprising incubating lymphocytes with a lymphokine to produce LAK cells and, exposing the lymphocytes to a suitable antigen of said infectious agent. Preferably the lymphocytes are exposed to the antigen after activation and, more preferably the antigen is an infectious agent such as a virus or a retro-virus; the virus or retro-virus may be alive, dead, or in some way disabled; the antigen may also be a virus particle or a protein. The lymphocytes for

treatment may be derived from a healthy donor, not infected by said infectious agent, or from a suitable cell line.

In a more preferred embodiment of the twelfth aspect of the present invention, the lymphocytes for activation are exposed to the antigen prior to activation, to thereby become capable of controlling said agent.

Advantageously, activated and stimulated lymphocytes produced by a process in accordance with the present invention, may be infused into a patient infected with the infectious agent, wherein the stimulated LAK cells act to reduce the level of infection within the patient. Such an infusion is particularly effective where the infectious agent has a harmful or debilitating effect on a patient who alone, is unable to react sufficiently to the presence of the infectious agent.

In a preferred embodiment of the twelfth aspect of the present invention, the infectious agent is HIV and the antigen is live HIV, inactive HIV, or a portion

thereof. The use of lymphocytes, stimulated and activated in vitro by a method of the present invention to react to HIV, is particularly useful in the treatment of AIDS. An AIDS sufferer's own immune system is damaged through Helper cell depletion and is unable to reduce the level of HIV infection to thereby recover some of its function. However, if lymphocytes processed in accordance with the present invention to respond to the HIV strain infecting a particular AIDS sufferer, are infused into the sufferer, the level of HIV infection may be reduced by the activity of the stimulated and activated lymphocytes.

In a further embodiment of the twelfth aspect of the invention, the lymphocytes for activation are derived directly or indirectly from a human or animal infected with said infectious agent, wherein said human or animal exhibits little or none of the deleterious symptoms of infection. The infectious agent may be a virus, is preferably a retro-virus and most preferably HIV.

In a thirteenth aspect, the present invention provides antibodies capable of controlling an infectious agent by neutralisation, which antibodies are harvested from lymphocytes previously exposed to an infectious agent and capable of controlling said agent.

Antibodies in accordance with the thirteenth aspect of the present invention may be employed in the same manner as LAK cells in accordance with the twelfth aspect of the present invention, in order to alleviate the symptoms of a patient suffering from the effects of infection with an infectious agent.

In an embodiment of the thirteenth aspect of the invention, the antibodies are derived either directly or indirectly from a human or animal infected with said agent, but exhibiting little or none of the deleterious symptoms of infection. The infectious agent may be a virus, is preferably a retro-virus and most preferably HIV.

In a fourteenth aspect, the present invention provides a therapeutic composition, comprising cells in accordance with the twelfth aspect of the invention,

antibodies in accordance with the thirteenth aspect of the invention, or a mixture of said cells and said antibodies.

It is important, in order that a therapeutic composition in accordance with the present invention is to be useful in treating a particular patient, for the lymphocytes used to have been exposed to the same infectious agent as that afflicting the patient, or for the antibodies to be specific for this agent. Thus, in treating AIDS or HIV infection, the lymphocytes must have been exposed to the same strain of the virus as that which afflicts the patient, or the antibodies must be specific for said strain. This is easily achieved by obtaining lymphocytes or antibodies, for activation, from an HIV positive individual who is without AIDS symptoms and, who is infected with the same strain of HIV as the AIDS sufferer it is intended to treat. For example, lymphocytes or antibodies, for activation, may be obtained from serum derived from the sexual partner of the patient, or from a parent to treat a child. Alternatively, the serum may be drawn from an individual during an early phase of HIV infection, or whenever an individual is not exhibiting AIDS symptoms,

and used to provide LAK cells, or activated antibodies for treating the same individual, when he is exhibiting AIDS symptoms. The serum may be stored in a frozen state until it is required.

The stimulated and activated lymphocytes act both directly on the virus by destroying lymphocytes (helper cells) which are expressing HIV and also, by activating the cytotoxic cells within the patient's circulation to attack both opportunistic infections and HIV expressing lymphocytes. The cytotoxic cells (CD8) of an infected individual are not damaged or infected by HIV, because they lack CD4 receptor sites, but they require activation by Helper cells before being able to respond to the presence of infection. It is for this reason that the cytotoxic cells of a person with AIDS do not respond to HIV or other opportunistic infections.

Thus the sufferer's immune sytem recovers some of its function, thereby alleviating some AIDS symptoms.

In an embodiment, where the lymphocytes are activated before exposure to a suitable antigen, the antigen is derived from serum extracted from a patient infected with the infectious agent it is intended the activated lymphocytes should respond to. Advantageously, the serum is extracted from the patient it is intended to treat and said serum includes the virus HIV.

Additionally, the serum may be extracted from the patient and HIV isolated from a culture of lymphocytes derived from the serum and thereafter used to stimulate donor lymphocytes. Preferably, prior to use, the HIV is exposed to ultraviolet light for between 24 and 48 hours to inactivate the virus. Alternatively, the virus may be heated at 56°C for 1/2 hour.

In a fifteenth aspect, the present invention provides a process for activating lymphocytes which have been exposed to an infectious agent comprising incubating the lymphocytes with a lymphokine to produce LAK cells. Preferably the infectious agent is a retro-virus, such as HIV.

In an embodiment of the fifteenth aspect of the invention, the lymphocytes for activation are derived from an individual infected with HIV and, during activation, the lymphocytes are treated with a drug which inhibits viral replication, such as AZT.

In a sixteenth aspect, the present invention provides activated and stimulated lymphocytes produced by a process in accordance with the twelfth or fifteenth aspects of the present invention.

In a seventeenth aspect, the present invention provides therapeutic compositions comprising activated and stimulated lymphocytes produced by a process in accordance with the twelfth or fifteenth aspects of the present invention.

In embodiments of the present invention the genetic material within the lymphocytes is modified after activation so as to render virus production by the lymphocytes impossible. This may be achieved by treating the LAK cells of the invention with a suitable chemical composition such as a DNA inhibitor or with

radiation. A suitable DNA inhibitor is the drug mitomycin-C.

In an eighteenth aspect, the present invention provides a method of producing LAK cells comprising obtaining lymphocytes, either directly or indirectly, from an individual infected with an infectious agent who exhibits substantially no deleterious symptoms of such infection, and activating said lymphocytes by incubation with a suitable lymphokine. In an embodiment the infectious agent is a virus, preferably a retro-virus and more preferably HIV.

In a preferred embodiment of this further aspect of the present invention, the lymphocytes or antibodies are treated with a substance to remove substantially all free infectious agent, or to protect activated cells from infection with the infectious agent. In the case where the infectious agent is HIV, the substance employed may be AZT (azidothymidine) or dextran sulphate.

In a nineteenth aspect, the present invention relates to a method of treating an individual infected with an infectious agent comprising, administering lymphocytes activated and stimulated to respond to said infectious agent in accordance with the the present invention.

It has been determined that donor LAK cells promote increased suppressor cell activity, which in turn casues a patient's ability to respond to foreign cells to be reduced. Nevertheless the donor LAK cells maintain an ability to kill the patient's malignant, or virus infected cells.

Accordingly, the twentieth aspect of the present invention provides a composition for use in a method of increasing suppressor cell activity, which composition comprises activated lymphocytes from a patient, or donor prepared by incubating a body liquid containing autologous or donor lymphocytes with a lymphokine.

This composition of the invention may be used to suppress patients' ability to respond to foreign cells and to thereby induce a tolerance to the presence of foreign tissue. Thus the composition of the twentieth

aspect of the present invention is usable in transplant procedures in order to reduce the incidence of rejection of transplanted tissue. This usefulness applies to the transplantation of both heamopoietic tissue and other organs such as kidneys, livers, and hearts.

The invention will now be described, by way of illustration only, with reference to the following Examples:

Example 1

Autologous lymphocytes were collected from a patient by lymphopheresis using a cell separator to give between 2.5 and 10 x 10^9/1. The volume of fluid collected during pheresis varies between 200 and 400 ml, the proportion of lymphocytes varying between 55 and 70% since, of course, other cells are collected by this process.

Alternatively, where pheresis is impractical 500 ml of blood may be taken from the patient by straight venesection.

The suspension is now incubated for one hour at about 37 ^{o}C, without antibiotic or medium added. The process is conducted in 1 litre transfer packs produced by "Fenwall". In fact only 3-500 ml of the suspended cells are placed in each 1 litre bag. After the addition of Interferon Alpha and incubation for one hour, the entire material is reinfused into the patient.

Example 2

The process given in example 1 above was repeated such that the incubation with the Interferon Alpha had been completed.

The incubated body liquid products were then subjected to purification to give a lymphocyte population of 2.5-6.5 x 10^9/l. The lymphocytes are then separated by Ficoll-Paque to give an end percentage of lymphocytes of greater than 95 % having a viability of greater than 95%. The separated lymphocytes are then washed with Hanks Balanced salt solution and subsequently suspended in a culture medium RPMI 1640 supplemented with 5-10% of autologous plasma containing

INF and a suitable antibiotic, for example streptomycin and/or penicillin; 100 units of each.

The lymphocytes are suspended in the RPMI culture medium to a concentration of 1-3 x 10^9/1. 200 ml of the suspension is placed in an 850 Gibco tissue culture flask or Fenwall transfer bag.

These autologous cells disposed in the culture medium are cultured for 48 to 72 hours, separated by centrifugation at 350 g to separate the LAK cell containg supernatant. The process is then repeated for one hour at 3000 rpm (700 g) to produce platelet free supernatant.

The cells are centrifuged at 350 g force for 10 minutes and the consequent pellets are then collected, washed three times with Hanks Balanced Salt Solution which is supplemented with 5% autologous patients' plasma and 10 units of preservative-free heparin. The cells are finally suspended in 0.9 sodium chloride, 10 units of preservative-free haparin and 10% autologous plasma making the final volume of pre-infusion composition 200

ml. The volume of cells reinfused into the patient vaies between 0.3-7 x 10^9/l.

Concurrently with the foregoing procedure, donor cells are extracted from a healthy individual, for example, a family member of the patient. The donor cells are treated in precisely analagous form as the patient's cells with the exception, of course, that since the cells are healthy, there is no need to add AZT. After culture for 48 to 72 hours the donor cells are centrifuged in precisely the same way as the autologous cells, to provide healthy LAK cells containg the identifyable lymphokines, Interleukin 2, Interleukin 1 and Gamma Interferon. Other lymphokines are also present but these have not yet been identified.

In a further process the donor lymphocytes used for lymphokine production may be obtained by centrifugation of 50% of the culture medium at 24, 48 and 72 hours respectively.

Such donor LAK cells may be stored at -20 C or may be infused directly into the patient or added directly to the patient's cultured LAK cells in a volume of 1:4 or 1:2. This suspension may be directly added to the autologous LAK cells at any convenient period.

Alternatively the lymphokines from the donor LAK cells may be extracted and used to stimulate autologous lymphocytes.

Where the donor LAK cells are held separate from the autologous LAK cells they will need to be separated and purified in an analagous way.

Accordingly, the invention provides a method for activating LAK cells without fractionation, and a method for potentiating the action of LAK cells by addition thereto of donor LAK cells as defined.

Example 3

Allogeneic (donor) lymphocytes were administered to patients suffering from malignant disease and HIV infection. The donors were variously spouses and blood

relatives. In only one case was the donor HLA, MLR and blood group identical to the patient.

The lymphocytes were obtained by a process of lymphocytopheresis and further purified using ficol-paque. Activation of the lymphocytes was achieved by adding Interferon Alpha at 1000 units per millilitre. The activated lymphocytes were then incubated for between one and 72 hours and, on occasions, even longer. The activated lymphocytes were then administered to the patients. The incidence of fever was less than 10% of reinfusions. This is more or less the same incidence of fever as seen in reinfusion of autologous cells. No other side effect has been identified. In some cases, anti donor lymphocyte antibodies were identified after treatment, while in other no such antibodies were seen. It is considered that the patients had a prior degree of immune suppression because of their disease. In some cases the absolute lymphocyte numbers were normal.

It is considered that the patients' ability to respond to the presence of the activated donor lymphocytes was

suppressed by increased suppressor cell activity, induced by the activated donor lymphocytes.

Example 4

Autologous lymphocytes were collected from a patient by lymphopheresis using a cell separate to give between 2.5. and 10 x 10^9/1. The volume of fluid collected during pheresis varies between 200 and 400 ml, the proportion of lymphocytes varying between 55 and 70% since, of course, other cells are collected by this process.

Alternatively, where pheresis is impractical 500 ml of blood may be taken from the patient by straight venesection.

To either of these lymphocyte containing body liquids is added 1000 IU ml Interferon Alpha. Where HIV positive patients are being treated, 1-5 micromoles of AZT is also added.

To either of these lymphocyte containing body liquids is added 1000 IU ml Interferon Alpha. Where HIV positive patients are being treated, 1-5 micromoles of AZT is also added.

The suspension is now incubated for one hour at about 37°C.

In this example of the invention, the process is conducted in one litre transfer packs produced by "Fenwall". In fact only 3-500 ml of the suspended cells are placed in each one litre bag. After the addition of Interferon Alpha and incubation for one hour, the entire material is reinfused into the patient.

Example 5

250 ml of buffy coat comprising 3-12 x 10^9 lymphocytes/litre was collected from a healthy donor by pheresis, using a cell separator HV50 (haemonetic). The buffy coat of cells so collected was treated with interleukin 2 (either commercially obtained or produced

The lymphocytes were then incubated for 1 hour with 1000 IU of interferon alpha (IFN).

The resulting activated cells were then treated with mitomycin-C at a concentration of 25-40 μg/ml. The cells were incubated with the mitomycin-C for 45 minutes at 37°C.

The modified activated cells were then washed 2-3 times and were prepared for infusion into an individual infected with HIV in 200 ml saline, supplemented with 10% autologous plasma.

Optionally, the lymphocytes may be modified through irradiation with between 300 and 3000 rads, in place of exposure to mitomycin-C.

Example 6

Lymphocytes were collected from the sexual partner of a patient suffering from AIDS, by lymphopheresis using a cell separator to give between 2.5 and 10 x 10^9 lymphocytes/Litre. Prior to lymphocyte collection, it

is established that the sexual partner, although not suffering from the symptoms of AIDS, is infected with HIV. The volume of fluid collected during pheresis varies between 200 and 400 ml, the proportion of lymphocytes varying between 55 and 70% since, of course, other cells are collected by this process. Alternatively, where pheresis is impractical, 500 ml of blood may be taken from the partner by straight venesection. To either of these lymphocyte containing body liquids 1000 IU alpha interferon is added together with 1-5 micromoles of AZT. This suspension was then incubated for one hour at about 37 °C. The process is conducted in a one litre transfer pack produced by "fenwall". In fact only 300-500 ml of the suspended cells are place in each one litre bag.

After the addition of alpha interferon and AZT and subsequent incubation, the entire material is infused into the patient suffering from AIDS.

Example 7

250 ml of buffy coat was collected by pheresis, from the HIV positive husband of an AIDS sufferer. The buffy coat comprised approximately 8-18 x 10^9

lymphocytes per Litre.

The buffy coat was activated with 1000 IU of interferon alpha (IFN) plus 10μmol of AZT. After 1 hour's incubation, the plasma was removed and the lymphocytes separated on ficoll and then cultured with 10μmol of AZT and 1000 IU of IFN, in a mixture of the previously isolated plasma and fresh donor plasma. This mixture was cultured for 3-5 days and then washed once before suspension in the husband's plasma and 200 ml of saline.

The patient was infused with this suspension and the treatment repeated five times.

After treatment, the patient's AIDS symptoms, caused by opportunist infections were considerably reduced and, in some cases, completely eliminated.

Example 8

The subjects were a mother and child, the child suffering from AIDS and the mother being HIV positive.

After treatment, the patient's AIDS symptoms, caused by opportunist infections were considerably reduced and, in some cases, completely eliminated.

Example 8

The subjects were a mother and child, the child suffering from AIDS and the mother being HIV positive.

Lymphocytes were collected from the mother in a buffy coat derived by lymphopheresis, using a cell separator. 250 ml of buffy coat was collected with a cell concentration of between 2.5 and 10 x 10^9 cells per litre. The proportion of lymphocytes varying between 55 and 70% of the cells, since, of course, other cells are collected by this process.

To the buffy coat, 1000 IU's of interferon alpha was added. The suspension was incubated 72 hours in a one litre fenwall transfer pack.

After incubation, mitomycin-C was added at a concentration of 25 μg/ml and the mixture was allowed to incubate for a further 45 minutes. The cells were

Example 9

Serum is drawn from a patient suffering from AIDS, lymphocytes from the serum are cultured and HIV is isolated from that culture. The HIV so isolated is then exposed to ultraviolet light for 24-48 hours to inactivate the virus. Alternatively, the virus may be inactivated by heating, or used in its live state.

Donor Lymphocytes are collected from a healthy individual by lymphopheresis using a cell separator to give between 2.5 and 10 x 10^9 cells/litre. The volume of fluid collected during pheresis varies between 200 and 400 ml, the proporation of lymphocytes varying between 55 and 70% since, of course, other cells are collected by this process. Aternatively, where pheresis is impractical 500 ml of blood may be taken from a donor by straight venisection.

To either of these lymphocyte containing body liquids 1000 IU of alpha interferon is added. The suspension is then incubated for three days in order to stimulate the lymphocytes.

Live or inactivated virus, derived in the manner set out above, is then added to the stimulted lymphocytes and the resulting mixture is incubated for a further five days. Prior to incubation the mixture of activated lymphocytes and live or inactivated virus is treated with AZT, or another antiviral drug, in order to protect activated lymphocytes from infection with virus. The mixture is then tested for cytotoxic and viral inhibitory effects before use. Thereafter, the stimulated and activated lypmohcytes may be infused into the patient from whom the HIV was originally derived, in order to reduce the amount of HIV in his circulation and to combat the effects, both directly and indirectly, of opportunistic infections.

Example_10

250 ml of buffy coat was derived from a patient suffering from AIDS related complex (ARC), by lymphopheresis. The total cell count was 8-13 x 10^9 cells per litre.

The buffy coat, so derived, was treated with 1000 IU's of interferon alpha (IFN) and 10 μmol of AZT. The resulting mixture was incubated for 1 hour and, thereafter, the supernatant was removed.

The lymphocytes were then separated out from the remainder of the mixture on ficoll and thereafter cultured with 10μmol of AZT, 1000 IU of IFN in a mixture comprising the supernatent derived as set out above plus 25% of a similar supernatant, derived from a healthy donor culture.

After culturing, the resulting mixture was suspended in 200 ml of untreated autologous plasma and saline, and treated with 10μmol of AZT for infusion into the patient suffering from ARC.

The patient was given a total of 10 such infusions and, his symptoms of ARC began to improve after the eighth infusion.

Example 11

Generation of killer cells in an autologous system.

Example 11

Generation of killer cells in an autologous system. The generation of such cells may be achieved by the following routine:-

1. Pheresis using an HV50 cell separator to give 250 ml of buffy coat with a cell concentration of 3-12 x 10^9 cells per litre.

2. Incubate the buffy coat with 1000 IU of IFN and 10μmol of AZT for 1 hour.

3. Remove the supernatant plasma and centrafuge to remove platelets (preparation A).

4. Separate the lymphocytes from the remainder of the mixture on ficoll.

5. Culture the separated lymphocytes in a fenwall culture bag in the presence of 10μmol of AZT, preparation A and 25% of the supernatant from a lymphokine activated culture obtained from a healthy donor. This mixture should be cultured for 3-7 days.

6. After culturing, the lymphocytes should be washed with Hanks solution and resuspended in untreated fresh autologous plasma and saline at a total volume of 200 ml.

7. Prior to infusion, the suspension is tested for viability, infection and functional activity against various target infections.

8. The suspension is then reinfused into the patient, preferably 8-12 times. On infusion, the suspension comprises 1-10 x 10^9 cells per litre.

Example 12

MATERIAL AND METHODS

Lymphopheresis

Autologous and allogeneic peripheral blood lymphocytes (PBL) were obtained by a standard pheresis programme using an Haemonetics V50. Acid citrate dextrose was

used as the anti-coagulant. Autologous Lymphocytes were harvested in 5-6 surges over a period of approximately 2 hours at any one collection. HIV+ and HIV- donor lymphocytes were similarly harvested. The total cell collection was on average 9 x 10^9/L. The volume collected varied between 250 and 400 ml. Lymphocytes were harvested variously on 5-13 occasions at usually 3-day intervals.

<u>Lymphocyte purification</u>

Lymphocytes were separated on Ficoll Hypaque and washed with Hanks Balanced Salt Solution (HBSS) free of calcium and magnesium. The gradient was centrifuged at 400g for 30 minutes. After centrifugation the platelet rich plasma was discarded. Lymphocytes at the plasma Ficoll Hypaque interface were collected and washed in HBSS three times. Ficoll separation and cell harvesting were performed under sterile conditions.

Lymphocytes were cultured in RPMI 1640 supplemented with 10% autologous plasma. Interferon was added at a final concentration of 100-1000 IU/ml. The cells were cultured in the presence of AZT in a concentration of 10μ mols for 3-10 days. The cells were washed in HBSS and resuspended in 200 ml of normal saline with 10% plasma, prior to infusion or reinfusion into the patients.

CD4 Purification
as a Target for HIV Virus

Peripheral blood lymphocytes were collected as previously described. These cells were processed again as described above, however the cells prepared for culture had added phytohaemagglutinin (PHA) in a concentration of 10 μg/ml and interleukin 2 (IL2) 200 units/m. The final cell concentration of 1 x 10^6/ml was cultured in plastic Petri dishes over 1 hour at 37 $^\circ$C. Adherent cells were left behind in the Petri dish.

Further incubation of the cells took place in 5 ml syringes packed with nylon fiber. After 1 hour's incubation at 37 °C. the non-B cells were eluted with RPMI 1640. The eluted cells were then treated with monoclonal antibody to CD8 and complement. The purification of CD4 cells was of the order of 85%.

Infection of CD4 Population

The purified population of CD4 cells was mixed with HIV+ plasma to give a concentration of 1 x 10^6 /ml. The culture was further diluted with an equal volume of RPMI.

The cells were cultured in tissue culture flasks for 7 days at 37 °C. in the presence of 100 i.u./ml of IL2.

The infected cell collection was used as a target for cytotoxic T cells in a short ^{51}Cr release assay.

Virus Monitoring In Culture

HIV antibody and antigen were assessed by end point titration methods with Eliza (Abbott). To test for secondary infection, the supernatant from infected cultures was introduced into non-infected lymphocytes in culture.

On Day 11 cytopathic effects consisting of sincytium formation, cell enlargement and cell death were evaluated in triplicate.

Cell viability was identified by Trypan Blue dye exclusion and increase in the absolute number of cultured cells.

Cytotoxic Assay

Target cell line (K562) was labelled with 100 microcuries of sodium chromate. The cells were incubated at 37 ^{o}C. for 45 minutes. After incubation the cells were then washed 3 times and re-suspended in RPMI to give a final volume 3 x 10^{6} /ml. Effect of cell cytotoxicity was tested at 30:1 effector: target ratio. Culture in triplicate in round bottom 96 well

microtitre plates was performed at 37 °C. in 5% CO_2 for 4 hours. ^{51}Cr release was counted in a gamma counter. The results were expressed as a percentage of lysis using the formula, (% lysis = experimental CPM - spontaneous CPM/maximum CPM - spontaneous CPM) x 100. *

Autologous blast target cells were generated by incubation of the patient's blood lymphocytes in the presence of PHA 10 μg/ml and interleukin 2, 1000 IU/ml for 72 hours. The cells were infected with HIV on Day 3 and Day 5 and labelled with ^{51}Cr sodium chromate as above. The infected cells were tested for functional activity against target cells.

* CPM = counts per minute.

Immunologic Monitoring

Circulating levels of various lymphocyte subsets were monitored throughout the course of treatment.

CD4 and CD8 numbers were determined using monoclonal antibody conjugated to fluorescein by a technique of indirect immunafluorescence.

Differences in the lymphocyte subsets were observed between blood samples collected pre and post treatment.

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6 patients, 3 with AIDS and 3 with AIDS Related Complex (ARC) were treated with lymphokine activated killer (LAK) cells generated in vitro from autologous and allogeneic peripheral blood lymphocytes.

The lymphocytes were treated in vitro variously with interferon (IFN), interleukin 2 (IL2), azidothymidine (AZT) and mitomycin-C.
Approximately 1×10^9 cells were infused on each occasion, usually 3 days apart, and to a maximum total of 13 reinfusions.

Significant improvements were noted on a variety of parameters, including clinical, virologic and haematologic. Importantly, no immunologic deterioration was identified.

In addition, apart from an incidence of fever in less than 10% of reinfusions, no significant toxicity was identified.

Three different protocols were employed. Protocol selection was dependent upon the patient's condition and disease stage at presentation.

Protocol 1 (autologous cells) was used for the treatment of patients with ARC. These patients had white cell counts $>4 \times 10^9$ /L and CD4 $> 0.5 \times 10^9$ /L.

Protocol 2 (HIV+ allogeneic cells) was used in the treatment of a patient with AIDS. The white cell count was $<4 \times 10^9$/L, CD 4 $> 0.04 \times 10^9$ /L and CD 8 $< 0.8 \times 10^9$/L. The patient selected for this protocol was infected with the same strain of HIV as her husband who was used as the donor. This protocol could be used in vertically transmitted infection in addition to horizontally transmitted disease. The donor was asymptomatic. The purpose of this protocol was to overcome the problem of very low numbers of CD4 cells in stage 5 and 6 disease. Furthermore, patients with

late stage disease, beacuse of leukopenia and debility, make them poor candidates for leukapheresis.

The possible benefit of giving activated immune HIV+ cells from an asymptomatic donor to an AIDS suffering wife might also be supplmented by using plasma at the same time.

Protocol 3 (allogeneic activated cells treated with mitomycin-C) was used in AIDS sufferers, the donors being healthy individuals. Cells for infusion were treated with mitomycin-C to prevent virus infection and replication in donor cells. Although DNA damage was to be anticipated, it was considered that the cells would retain the ability to bind the free HIV antigen and that the release of CD4 signals would remain intact.

Donor selection was usually made among family members, although identity was not considered to be important.

Brief description of the figures and tables:

Table 1 sets out the clinical features of the six subject patients;

Table 2 shows immunoconversion of all six subject patients before and after treatment;

Table 3 shows the cytotoxicity induced by LAK cells from PBL of patients 1 and 2, before treatment and after 2-4 treatments;

Table 4 shows the lymphocyte count for patient 6 during treatment;

Table 5 sets out the cell viability for patients 1-4 and 6 before treatment and after 5 infusions and;

Table 6 sets out the antigen titer before, during and after treatment for patients 3-5.

Figure 1 shows the rate of syncytium formation in cultures taken from patients 1-3 before treatment;

Figure 2 shows the increase in the number of syncytia present in a 4 day old culture taken from patients 1-3 prior to reinfusions 1-4;

Figure 3 shows the T-cell subsets in patient 4 before treatment and after 1-10 treatments;

Figure 4 shows the T-cell subsets in patient 3 after treatment with HIV+ and normal allogenic activated cells;

Figure 5 shows the lymphocyte subsets after treatment with eight infusions (Patient 2);

The effects of treatment was monitored in a variety of ways. In culture, HIV+ cells express the envelope glycoprotein GP120 which produces cell fusion and sincytium formation of both infected and non-infected cells. Ultimately, cell death occurs among all cells bearing CD4 molecules.

Before treatment, mononuclear cells obtained by pheresis and cultured in the presence of interferon 100-1,000 i.u. /ml. and AZT 5μ mols were clumped together to form nucleated giant cells which reached a peak of 65-70% of cells in culture on day 4 in patients 1 and 2. On the other hand, patient 3 had little increase in sincytail formation in culture, See figures 1 and 2.

The differences between patients 1 and 2 and patient 3 may be attributable to the concentrations of the AZT used.

Alternatively, patients 1 and 2 may have reached the more advanced stage of the disease. Furthermore, variation in virulence of the strains of virus among these patients could not be excluded.

Culture of cells analysed after the first, second and third reinfusions and grown for 4 days showed very few sincytia.

Cell death and culture was high before treatment, being greater than 58% after three days growth in vitro. Following treatment, this was markedly reduced. See table 5. Further, the cells looked more normal with very few blast forms identifiable.

HIV antigenaemia was identified in the three AIDS patients before treatment with activated cells, while the three ARC patients were HIV antigen negative. In two of the ARC patients tested one year after the initial treatment, antigen negative status was maintained. See table 2.

Antigen was detected at a level of 1 in 16 in patient 4 before treatment with activated cells. After one reinfusion, antigen was not detected and this remained so until the end of treatment. Patient 5 had an antigen titre of 1 in 8 at presentation. This reduced to 1 in 2 after one infusion, and this patient remained antigen positive throughout treatment. Patient 6 was antigen positive and remained antigen positive when treatment was aborted after three reinfusions. See table 6.

Culture of isolates from plasma and lymphocytes of patients 4 and 5 proved to be highly infectious with the death of about 70% of the cultured cells after five days. The reduction of cell death in culture shown in these patients after treatment suggests a reduction in the level of virion. See table 5.

Culture of PHA and IL2 activated autologous lymphocytes in the presence of normal lymphocytes from patients 1 and 2 after 9-12 days resulted in cell infection. On completion of treatment and culture, no evidence of infection was found.

Cytotoxic NK (normal killer) and T-cell activity was improved following treatment (Table 3). This was identified using K562 targets where, in patient 1, post treatment cytoxicity was 35% higher than pre treatment and 23% higher in patient 2. A similar effect was seen with HIV infected blasts where the increase was 29% for patient 1 and 17% for patient 2.

The generation of activated cells in HIV infection presents difficulties in that such cells are more susceptible to HIV infection than resting cells. To

overcome this problem, we have endeavoured to protect activated cells from infection by exposing them in vitro to AZT and interferon . AZT is effective against HIV replication at the stage of reverse transcription, whereas interferon is effective against viral budding.

The HIV virus particularly infect CD4 cells. The infected cells express the HIV envelope glycoprotein GP120, which induces cell fusion, sincytium formation and ultimately cell death. The initially high level of cell death and sincytium formation was shown to be reduced in vitro in the presence of AZT and interferon.

The addition of interleukin 2 to interferon produced a striking augmentation in the numbers of NK cells and CD8 cells. These cells were capable of inducing lysis in Nk sensitive targets (K562) and HIV infected targets. Cytotoxicity was increased to twice that (see treatment table). The combination of interferon , interleukin 2 and AZT in vitro suggests that this combination will be relevant to in vivo management.

CD8 numbers were increased in the circulation following treatment (figure 5 and table 4). The enhancement in cytotoxicity which we witnessed could be due to this increase, and this may play a significant role in preventing HIV infection of other cells. It is probable that the CD8 cells kill virally infected cells and neutralise free antigens.

P24 antigen has been used in other clinical studies to monitor the effect of HIV inhibitory agents. The level of antigen in patients 4 and 5 dropped from 240 to 90 picograms/L in less than 10 days and following 1 reinfusion. Clinical studies with AZT have shown the same reduction in antigen level, but only after 16 weeks.

Five of the 6 patients treated showed improvement in a number of clinical parameters. see table 1. The resolution of fever and sweats was striking and dramatic. The resolution of lymphadenopathy of patients 1, 2 and 3 was reasonably prompt. The improvement in appetite and weight gain seemed again to relate to the treatment. The weight gain, in particular, was not related to fluid retention.

The improvement in cerebration and locomotion in 2 of the AIDS patients again occurred during the treatment phase and again compared favourable with the deterioration in these features while the patients have been receiving AZT.

The improvement in ulceration and the various infections again appeared to specifically relate to treatment. The lack of side effects, in particular the low incidence of fever, is an important advantage, of the treatment with activated cells.

TABLE 1

CLINICAL FEATURES IN THE SIX PATIENTS SUFFERING WITH ARC AND AIDS
AFTER TREATMENT WITH ACTIVATED CELLS

Clinical Features	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Anorexia	Improved	Improved		Improved	Improved	
'eight Gain	2.5 kg.	2 kg.	2 kg.	5 kg.	4.5 kg.	
Fever, Sweats, etc.	Disappeared	Disappeared	Disappeared	Disappeared	Disappeared	
Diarrhoea				Improved		
Lymphadenopathy	Disappeared	Disappeared	Improved	Not present	Not present	Not prese
General Malaise	Improved	Improved	Improved	Improved	Improved	
Infections	Oral candad-iasis not present Penile lesions improved	Oral candad-disappeared Penile lesions not present	Oral candad-iasis not present Penile lesions not present	Oral candad-iasis not present	Oral candad-iasis not present	Oral candad-iasis not present Penile lesi not present
Tinea	Not present	Not present	Disappeared	Disappeared	Not present	Not present
Cryptococcosis	Not present	Not present	Not present	Not present	Not present	Unchanged
Herpes - hand, mouth, vagina	Not present	Not present	Not present	Complete improvement	Complete improvement	Not present
C & V Infection (Retinitis)	Not present	Not present	Not present	Partial improvement	Partial Improvement	Not present
Neurological Disorders (walking & memory)	Not present	Not present	Not present	Partial improvement	Partial improvement	Not present
Malignancy	Not present	Not present	Not present	Not present	Not present	Recurrent Kaposi's

TABLE 2

IMMUNOCONVERSION IN AIDS & ARC

PATIENTS AFTER IMMUNOTHERAPY

Diagnosis		At Presentation		After AI Treatment	
		Ag	Ab	Ag	Ab
(1)	ARC	(-)	(+)	(-)	(+)
(2)	ARC	(-)	(+)	(-)	(+)
(3)	ARC	(-)	(+)	(-)	(+)
(4)	AIDS	+	+	(-)	(+)
(5)	AIDS	+	+	(-)	(+)
(6)	AIDS	+	+	(Not tested)	(+)

Six patients (3 AIDS & 3 ARC) were tested for Ag p24 at presentation and at the end of the treatment. All 3 ARC patients remain Ag(-) after 1 year of treatment. Interestingly, sera of two of the AIDS patients Ag+ converted to Ag(-) 1-2 months after treatment.

TABLE 3

Cytotoxicity induced by LAK cells from PBL of ARC patients1 and 2

Ratio effector: Target cells 30:1

TARGET CELL K562: (nonspecific)

	^{51}Cr release			
	0 time	Second pheresis	Third pheresis	Fourth pheresis
Patient 1	22% ± 3	18% ± 7	35% ± 12	57% ± 15
Patient 2	17% ± 5	20% ± 2	32% ± 8	40% ± 10

AUTOLOGOUS BLAST: (specific)*
(HIV infected)

Patient 1	9% ± 5	10% ± 4	25% ± 3	38% ± 9
Patient 2	15% ± 6	13% ± 3	21% ± 5	32% ± 12

Mean ± SD

* Induced by PHA treatment

TABLE 4

Patient (6)
Diagnosis

ARC T-lymphocytes Subsets in peripheral blood

	Total lymphocyte	pan T	T4	T8	Ratio T4/T8
	Absolute Count x 10^9/L				
Before treatment 6th June	2.49	2.241 (90%)	0.649 (29%)	1.008 (45%)	0.64
Post AZT treatment & before A.I. therapy		1.387 (75%)	0.345 (25%)	0.527 (38%)	0.65
A.I. treatment 22nd September		1.53 (85%)	0.306 (20%)	0.795 (52%)	0.38
7th October	2.48	2.1 (88%)	1.06 (43%)	0.942 (38%)	1.13
17th October	2.68	2.19 (82%)	0.591 (27%)	1.314 (60%)	0.45

TABLE 5

Cell viability of normal PBL in culture infected with HIV isolates

Patient No		Before treatment % cell viability inculture	Sources of isolation	After 5 treatments % cell viability inculture
1	(ARC)	48 ± 12	(supernatant)	70% ± 12
2	(ARC)	40 ± 8	(supernatant)	82% ± 20
4	(AIDS)	35 ± 7	(plasma)	55% ± 17
5	(AIDS)	29 ± 5	(plasma)	52% ± 11
3	(ARC)	65 ± 15	(supernatant)	85% ± 17

CLAIMS

1. A method for the activation of lymphocytes from a patient or donor for use in adoptive immunotherapy characterised in that autologous or donor lymphocyte containing liquid is incubated with a lymphokine whereby the resultant culture containing LAK cells is returnable direct to the patient.

2. A method according to claim 1 characterised in that the body liquid is lymphocytes collected by lymphopheresis or whole blood.

3. A method according to either preceding claim characterised in that Azidothymidine is added to the body liquid prior to incubation.

4. A method of activating autologous lymphocytes for infusion into a patient in adoptive immunotherapy, which method comprises incubating said autologous lymphocytes with a lymphokine to give LAK cells, characterised in that donor allogenic lymphocytes are incubated with a lymphokine and the admixture of

autologous and allogenic LAK cells is prepared for reinfusion into the patient.

5. A method of activating donor lymphocytes for infusion into a patient in adoptive immunotherapy, which method comprises incubating a donor allogenic lymphocyte containing liquid with a lymphokine to give LAK cells; characterised by preparing said LAK cells for direct infusion into the patient.

6. A method according to claim 4 or 5 wherein lymphocytes are fractionated after incubation with the lymphokine.

7. A method according to claim 4 characterised in that the autologous and the allogenic LAK cells are cultured subsequent to the incubation step.

8. A method according to claim 7 characterised in that the cultured allogenic LAK cells are added to the autologous LAK cells prior to the culture of the autologous LAK cells.

9. A method according to claim 4 or 5 characterised in that the lymphokine is selected from Interferon Alpha, Interferon gamma, Interleukin 2 or Interleukin 2 produced by phytohaemagglutinin.

10. A method as claimed in claim 9 characterised in that the lymphocytes are incubated with interferon alpha and interleukin 2.

11. A method according to claim 10 characterised by the addition of AZT.

12. A reinfusable therapeutic composition for infusion into a patient in adoptive immunotherapy, comprising an autologous or allogenic lymphocyte containing body liquid activated by a lymphokine in accordance with a method as claimed in any of claims 1-11.

13. A therapeutic composition for infusion into a patient in adoptive immunotherapy, which composition comprises autologous lymphocytes activated with a lymphokine to form lymphokine activated killer cells (LAK), in admixture with LAK cells formed by the

activation of donor allogenic lymphocytes activated with a lymphokine.

14. A therapeutic composition according to claim 13 additionally comprising an amount of AZT.

15. A method of treating a human or animal patient suffering from an infectious or oncological disease, comprising infusing lymphocytes activated by a method as claimed in any one of claims 1-11 into the patient.

16. A method as claimed in claim 15, wherein the infectious disease is acquired immune deficiency syndrome.

17. A method of activating and modifying cells having binding sites for infectious agents, which method comprises treating said cells with a lymphokine to provide lymphokine activated cells and, subsequently, modifying the activated cells so that the cells do facilitate effective replication of the infectious agent, while maintaining the cells' ability to bind said infectious agent.

18. A method as claimed in claim 17 wherein each of said binding sites provides a site for virus attachment and the activated cells are modified so as to facilitate virus reproduction.

19. A method as claimed in claim 18 wherein the virus is HIV and the receptor sites are preferably CD4 sites.

20. A method as claimed in any of claims 17-19 wherein the cells for activation and modification are lymphocytes.

21. A method as claimed in claim 18 wherein the activated cells are modified by the destruction or disruption of their genetic material.

22. A method as claimed in claim 21, wherein the modification is achieved through the exposure of the activated cells to a chemical agent, which agent is preferably a DNA inhibitor.

23. A method as claimed in claim 22, wherein the DNA inhibitor is mitomycin-C.

24. A method as claimed in claim 21 wherein the modification is achieved by exposing the activated cells to radiation.

25. A method as claimed in claim 20 wherein the lymphocytes for activation are derived from a healthy uninfected donor.

26. A method as claimed in claim 20 wherein the lymphocytes are derived from a suitable cell line.

27. A therapeutic composition for use in treating a patient infected with an infectious agent, which composition comprises cells which have been activated and modified by a method as claimed in any of claims 17-26.

28. A therapeutic composition, for use in the treatment of HIV infection or AIDS comprising cells activated and modified by a method as claimed in any of claims 17-26.

29. A method of treating a human or animal patient infected with an infectious agent, which method comprises infusing a composition as claimed in claim 27 into said patient.

30. A method of treating a human patient infected with HIV, which method comprises infusing a composition as claimed in claim 28 into said patient.

31. A process for lowering the concentration of an infectious agent such as HIV, in in-vitro stored infected body fluid, comprising incubating said body fluid with a composition comprising cells activated and modified by a method as claimed in any of claims 17-26.

32. A process as claimed in claim 31, further comprising the step of removing the activated and modified cells from said body fluid, after said cells have become bound to at least a portion of said infectious agent.

33. A process as claimed in claim 32 wherein cell removal is effected by centrifuging.

34. A process for activating and stimulating lymphocytes to respond to an infectious agent, comprising incubating the lymphocytes with a lymphokine to produce LAK cells, wherein the lymphocytes are exposed to a suitable antigen of said infectious agent.

35. A process as claimed in claim 34 wherein the lymphocytes are exposed to the antigen after activation.

36. A process as claimed in claim 35 wherein the infectious agent is a virus and the antigen is a virus, or virus particle.

37. A process as claimed in claim 35, wherein the lymphocytes for treatment are derived from a healthy uninfected donor.

38. A process as claimed in claim 35, wherein the lymphocytes for treatment are derived from a suitable cell line.

39. A process as claimed in claim 34 wherein the lymphocytes for activation are exposed to the antigen prior to activation, to thereby become capable of controlling said agent.

40. A process as claimed in any of claims 34-39 wherein the infectious agent is HIV and the antigen is live HIV, inactive HIV, or a portion thereof.

41. A process as claimed in claim 34 wherein the lymphocytes for activation are derived directly, or indirectly from a human or animal infected with said infectious agent, wherein said human or animal exhibits little or none of the deleterious symptoms of infection.

42. A process as claimed in claim 41, wherein said infectious agent is a virus, preferably a retrovirus, and most preferably HIV.

43. A composition comprising antibodies capable of controlling an infectious agent and neutralising said agent which antibodies are harvested from lymphocytes

previously exposed to the infectious agent and capable of controlling said agent.

44. A therapeutic composition comprising activated cells produced by a process in accordance with any of claims 34-42, antibodies in accordance with claim 43, or a mixture of said cells and said antibodies.

45. A process as claimed in claim 35 or 36, wherein the antigen is derived from serum extracted from a patient infected with the infectious agent.

46. A process as claimed in claim 45 wherein the serum is extracted from the patient it is intended to treat and preferably, the serum includes the virus HIV.

47. A process as claimed in claim 46 wherein the serum is extracted from the patient and HIV isolated from a culture of lymphocytes, derived from said serum and, thereafter, used to stimulate donor lymphocytes.

48. A process as claimed in claim 47 wherein, prior to use, the HIV is exposed to ultra-violet light for

between 24 and 48 hours to inactivate the virus prior to its use to stimulate the donor lympyocytes.

49. A process for producing LAK cells, comprising obtaining lymphocytes, either directly or indirectly, from an individual infected with an infectious agent, which individual exhibits substantially no deleterious symptoms of such infection, and activating said lymphocytes by incubation with a suitable lymphokine.

50. A process as claimed in claim 49 wherein the infectious agent is a virus, preferably a retrovirus, and more preferably HIV.

51. A process as claimed in any of claims 34-42 and 45-50 wherein the lymphocytes or antibodies are treated with a substance to remove substantially all the infectious agent, or to protect activated cells from infection with said infectious agent.

52. A process as claimed in claim 51 wherein the infectious agent is HIV and the substance employed is AZT or dextran sulphate.

53. Activated and stimulated lymphocytes produced by a process as claimed in any of claims 34-42 and 45-50.

54. A therapeutic composition including activated and stimulated lymphocytes as claimed in claim 53.

55. A method of treating a human or animal body infected with an infectious agent, comprising administering to the patient a composition as claimed in claim 54.

56. A composition for use in a method of increasing suppressor cell activity, which composition comprises activated lymphocytes from a patient or donor, prepared by incubating a body liquid containing autologous or donor allogenic lymphocytes with a lymphokine.

57. A composition as claimed in claim 56 which comprises an autologous or donor allogenic lymphocyte containing body liquid activated by a lymphokine to provide patient-specific LAK cells.

58. A method of treating a human or animal body infected with an infectious agent comprising, obtaining a body liquid containing lymphocytes, incubating the lymphocytes with a lymphokine to produce lymphokine activated lymphocytes and, infusing the activated lymphocytes into the patient.

59. A method as claimed in claim 58 wherein the lymphocytes are exposed to an antigen of the infectious agent before infusion into the patient.

60. A method as claimed in claim 58 wherein the lymphocytes are exposed to an antigen of the infectious agent prior to activation.

61. A method as claimed in claim 59, wherein the lymphocytes are obtained from a donor infected with the infectious agent.

62. A method as claimed in claim 60, wherein the donor is the patient to be treated and the lymphocytes are autologous, or the donor is not the patient to be treated and the lymphocytes are allergenic.

63. A method as claimed in claim 61, wherein the lymphocytes are obtained from the donor when the donor is not exhibiting a deleterious effect assocated with the infection.

64. A method as claimed in any of claims 58-63, wherein the lymphokine is interferon alpha, interferon gamma, or interleukin 2.

65. A method as claimed in any of claims 58-63, wherein the infectious agent is HIV.

66. A method as claimed in claim 63, wherein the donor is HIV positive and the patient has AIDS or ARC.

67. A method as claimed in claim 64 or 65, wherein the lymphocytes are incubated with a lymphokine in the presence of AZT.

68. A method as claimed in claim 58, wherein the lymphocytes are exposed to the antigen of the infectious agent after activation with a lymphokine.

69. A method as claimed in claim 68, wherein said infectious agent is obtained from the patient to be treated and is used to produce a non-active antigen.

70. A method as claimed in claim 69 wherein said non-active antigen is a dead virus or a virus particle.

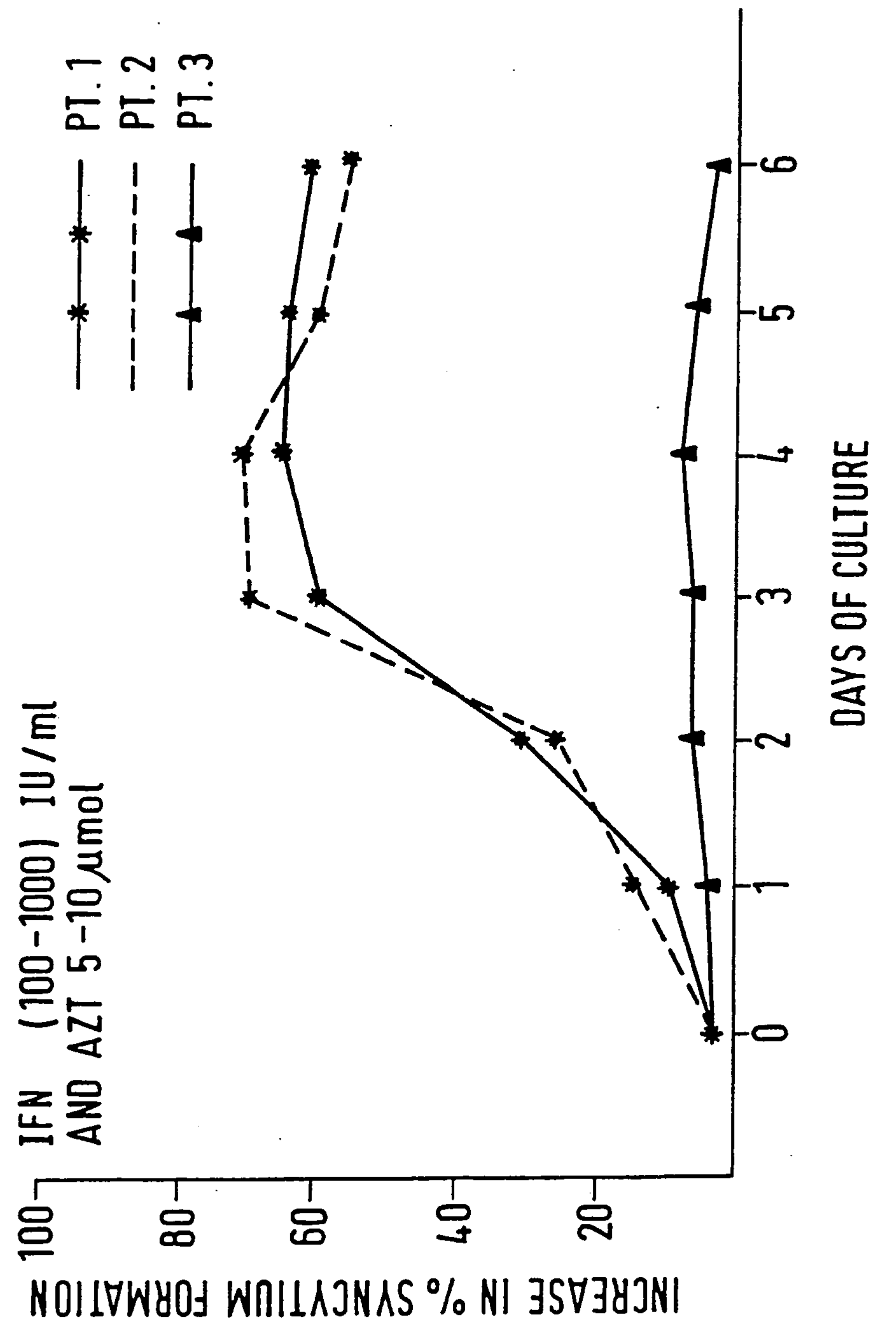
71. A method as claimed in any of claims 77-79 wherein, during exposure to the antigen, the lymphocytes are in the presence of AZT.

72. A method as claimed in any of claims 58-71, wherein the activated cells are treated with a DNA inhibitor, preferably mitomycin-C, prior to infusion into the patient.

73. A method as claimed in claim 72 wherein the lymphocytes are obtained by pheresis and thereafter purified by centrifugation.

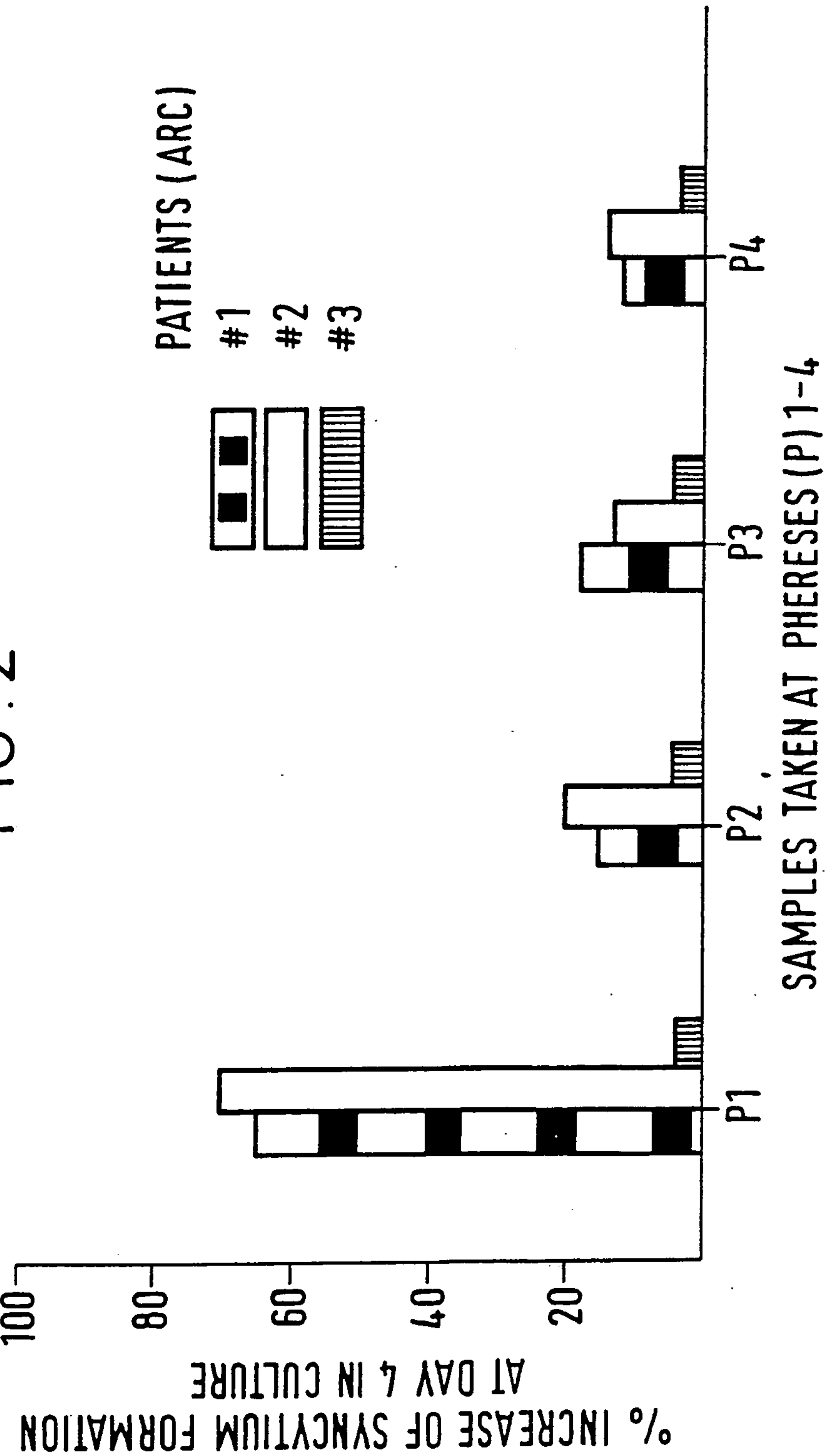
74. A method as claimed in any of claims 58-73 wherein the activated lymphocytes are LAK cells.

FIG. 1



SYNCYTIUM FORMATION IN CULTURE BEFORE TREATMENT

FIG. 2



SYNCYTIUM FORMATION IN CULTURE BEFORE AND AFTER TREATMENT

FIG. 3

T-CELL SUBSETS IN AIDS
(PATIENT 4) Ag+

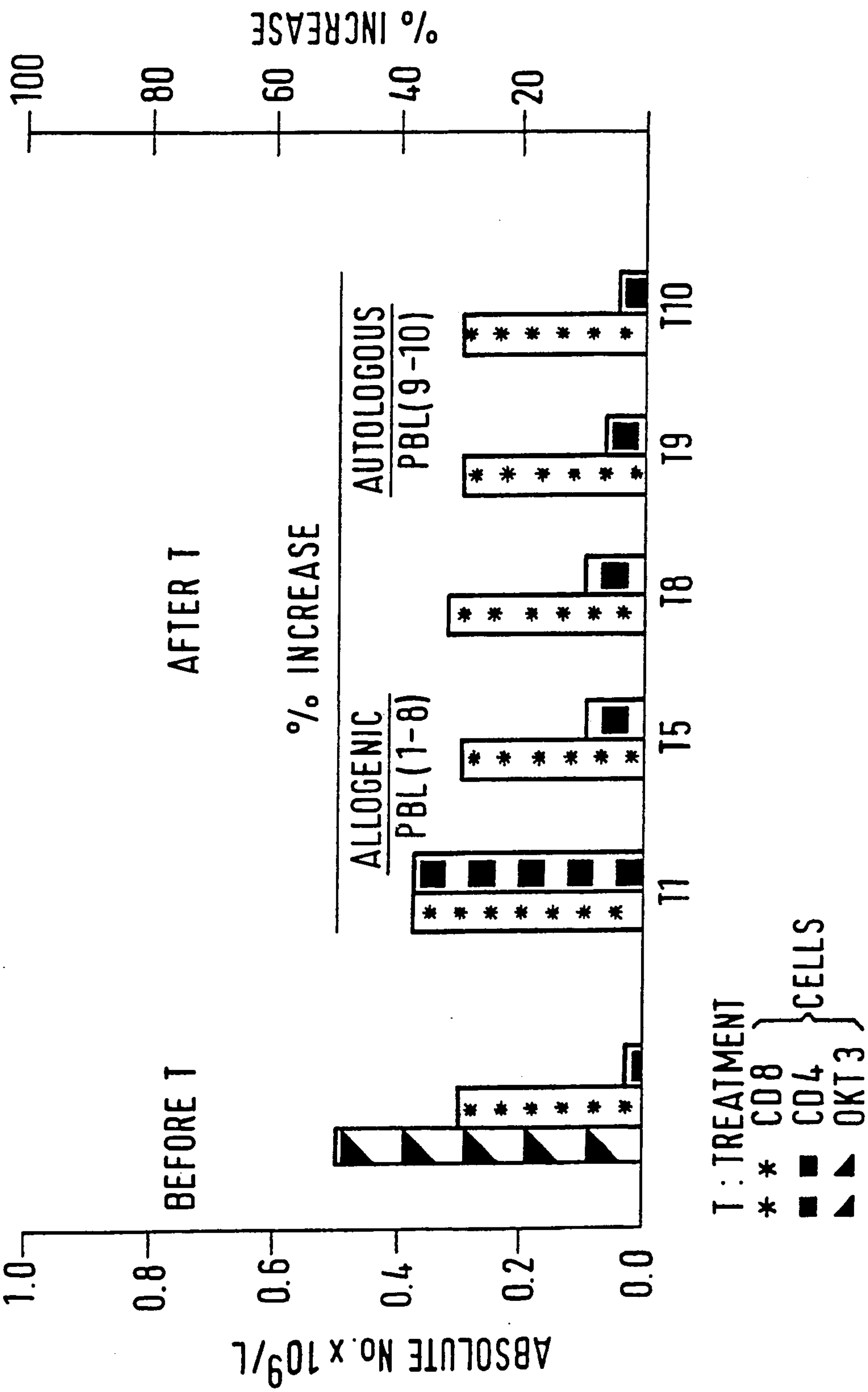


FIG. 4

T-CELL SUBSETS IN AIDS
(PATIENT 3) Ag+

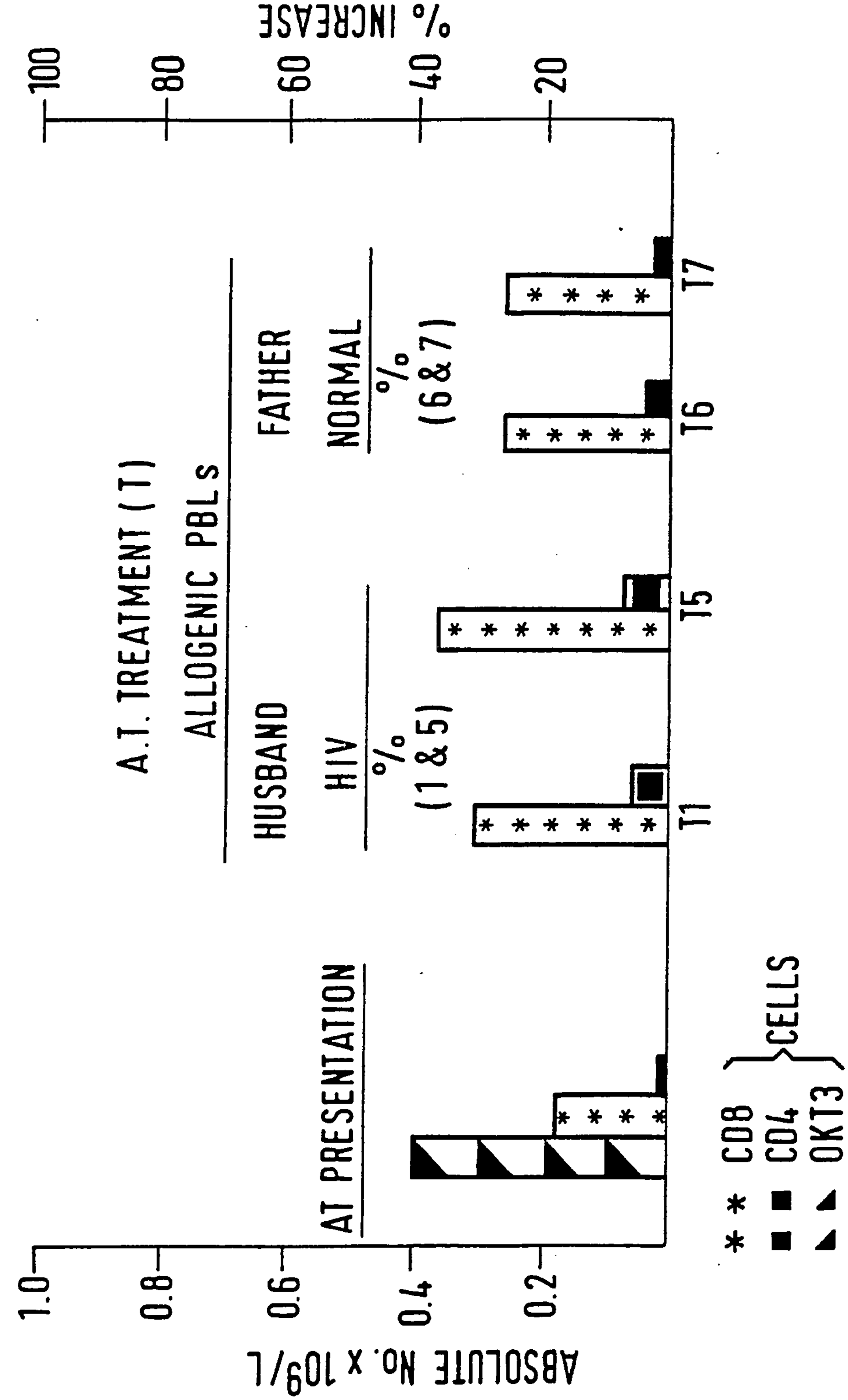
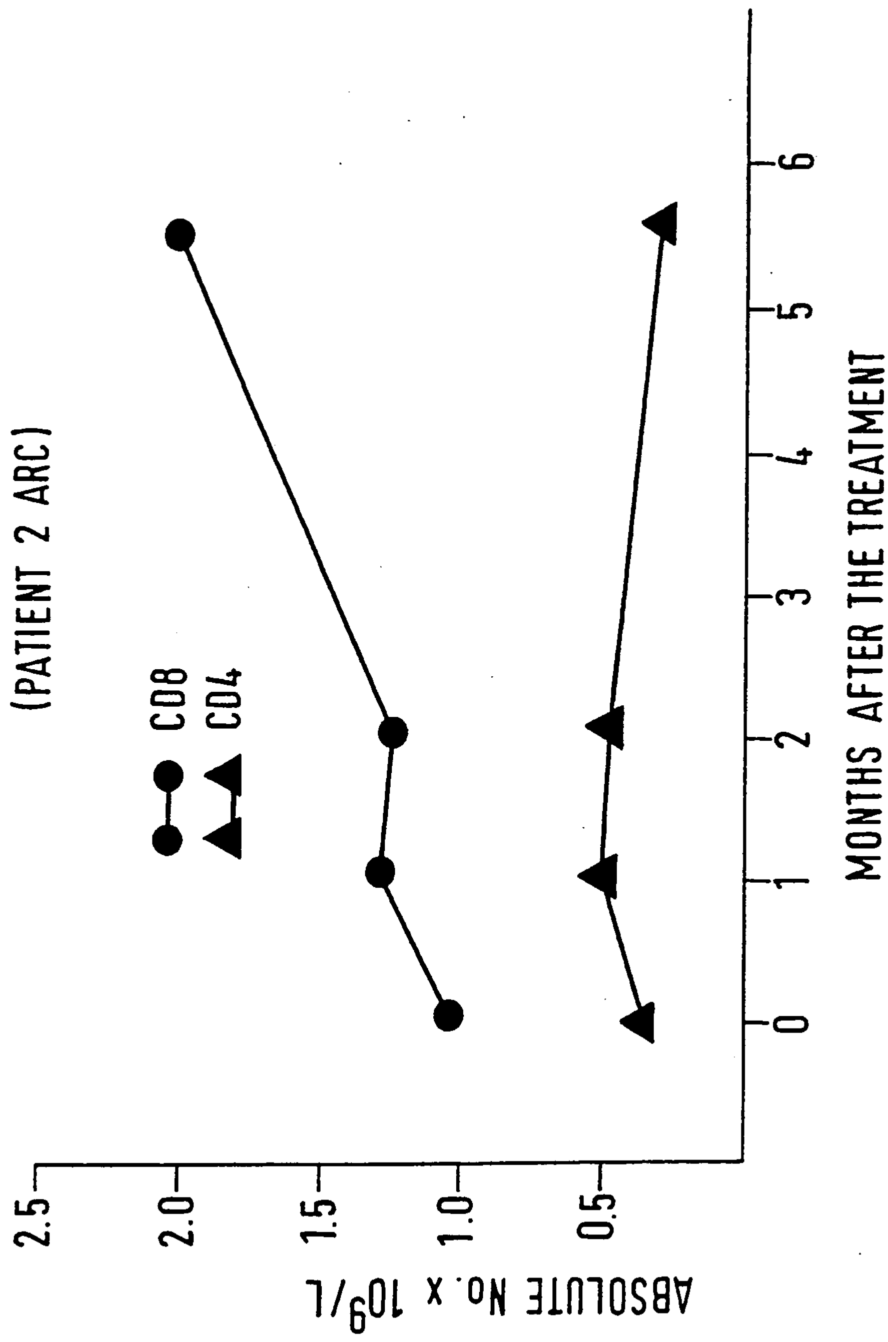


FIG.5

LYMPHOCYTE SUBSETS AFTER TREATMENT WITH LAK CELLS



LYMPHOCYTE PHENOTYPES WERE DETERMINED DURING AND AFTER 8 INFUSSIONS

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 88/01134

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC4: A 61 K 39/00, 39/21, 35/14, 45/02; C 12 N 5/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC4	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
P,X	EP, A2, 0 260 714 (ONCOGEN) 23 March 1988, see page 3 line 50 - page 7 --	34,39-42,49-50, 53,54
P,X	WO, A1, 88/07077 (THE CHILDREN'S HOSPITAL, INCORPORATED) 22 September 1988, see in particular page 11, line 30 - page 25, line 4 --	34,39, 41,49, 50
X	BIKEN JOURNAL, Vol. 30, 1987 Y. Kimoto et al.: "Adoptive immunotherapy of malignant diseases with IL-2-activated lymphocytes ", see page 29 - page 38 see in particular pages 29-31 and page 36 --	1,2,5,9, 10
X	WO, A1, 87/06610 (ENDOTRONICS, INC.) 5 November 1987, see page 5, line 8 and claims 3 and 7 --	9,10

[*] Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10th April 1989	25 APR 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	WO, A1, 87/00054 (THE REGENTS OF THE UNIVERSITY OF MINNESOTA) 15 January 1987, see pages 6-16 --	1,2,5,9
X	EP, A2, 0 211 769 (THE UNITED STATES OF AMERICA) 25 February 1987, see pages 7-9, page 30, lines 4-7 and claims --	1,2,5,9
X	CANCER RESEARCH, Vol. 46, June 1986 K. Itoh et al.: "Interleukin 2 activation of cytotoxic T-lymphocytes infiltrating into human metastatic melanomas ", see page 3011 - page 3017 see in particular pages 3011-3012 --	1,2,5,9
P,X	EP, A2, 0 257 962 (BECTON, DICKINSON AND COMPANY) 2 March 1988, see in particular claims 9-12 and pages 2-4 --	1,5, 12
P,X	WO, A2, 88/00970 (REGENTS OF THE UNIVERSITY OF MINNESOTA) 11 February 1988, see in particular page 5, lines 19-23 --	1,2,5,9, 34
X	GB, A, 2 106 935 (OTSUKA PHARMACEUTICAL CO. LTD.) 20 April 1983, see in particular page 3, left column, examples 1-7	1,5,9
Y		34-42,44 -50,53,54
	--	
X	Dialog Information Services, File 154: Medline 81-89/MAR, accession no. 05896715, Ruscetti F.W. et al. "Analysis of effector mechanisms against HTLV-I- and HTLV-III/LAV--infected lymphoid cells", & J Immunol May 15 1986, 136 (10) p3619-24 see the whole document	1,5,9
Y		34-42,44-50,53,54
	--	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Dialog Information Services, File 154: Medline 81-89/MAR, accession no. 06415462, Froelich C.J. et al.: "Lysis of human T cell leukemia virus infected T and B lymphoid cells by interleukin 2-activated killer cells", & J Immunol Dec 1 1987, 139 (11) p3637-43	1,5,9
Y	see the whole document	34-42,44-50, 53,54

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers ...*..... because they relate to subject matter not required to be searched by this Authority, namely:

   * 15,16,29,30,55, 58-74

2. [ ] Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers.........., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 88/01134

SA 26325

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 03/03/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0 260 714	23/03/88	JP-A- 63119428	24/05/88
WO-A1- 88/07077	22/09/88	AU-D- 13630/88	10/10/88
WO-A1- 87/06610	05/11/87	EP-A- 0302894	15/02/89
WO-A1- 87/00054	15/01/87	AU-D- 61254/86	30/01/87
		EP-A- 0227809	08/07/87
		JP-T- 62503144	17/12/87
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		US-A- 4690915	01/09/87
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		NL-A- 8202638	02/05/83
		JP-A- 59001420	06/01/84
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		AT-A- 377640	10/04/85
		DE-A-C- 3249568	07/02/85
		BE-A- 893704	18/10/82
		CA-A- 1195269	15/10/85
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		CH-A- 655661	15/05/86
		JP-A- 58059923	09/04/83
		CA-A- 1201988	18/03/86
		SE-A- 8204058	30/06/82
		JP-A- 58059922	09/04/83
		JP-A- 58057321	05/04/83
		JP-A- 58057318	05/04/83

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82